Errors in the evaluation of Arrhenius and van't Hoff plots

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Errors in the numerical values of activation or normal enthalpies, entropies and free enthalpies calculated from Arrhenius or van't Hoff plots, respectively, are due to the neglect of equidimensionality in equations, or to inappropriate approximations. The logarithmization of dimensioned quantities should be avoided, which demands the use of relative concentrations if a change in mole number occurs in the reaction. The application of the Arrhenius plot to enzymic reactions by using $V_{\max}/E_T$ instead of the rate constant of product formation has meaning only if the reaction follows the simplest Michaelis–Menten mechanism; however, the use of the van't Hoff plot using K_m is questionable even in the latter case.

The temperature dependence of the rate of enzymic reactions and their equilibrium constant are generally analysed by using the empirical Arrhenius equation ($\ln k = \ln A^0 - E_a/RT$) or the van't Hoff equation ($\Delta G^0 = -RT \ln K_{eq}$), respectively (van't Hoff, 1884; Arrhenius, 1889). In such an analysis mistakes can frequently be found due to the fact that authors do not adhere to the simple rules: (i) the two sides of an equation must have the same dimensions, (ii) only dimensionless pure numbers have logarithms or (iii) inappropriate approximations should not be used.

Arrhenius plots

First order reactions. These can be interpreted in terms of the absolute rate theory (Eyring, 1935). In this case the first-order rate constant (Benson, 1960; Laidler, 1963):

$$k = (\kappa RT/Nh) e^{\Delta S^\ddagger/R} e^{-\Delta H^\ddagger/RT} \quad (1)$$

Abbreviations and symbols used: A^0 , pre-exponential factor; A_1^0 , A^0/e ; k , rate constant; E_a , activation energy, $E_a \approx \Delta H^\ddagger + RT$; $H^\ddagger$ being the activation enthalpy; G^0 , normal free enthalpy (Gibbs function); K_{eq} , equilibrium constant; R , gas constant; T , absolute temperature; κ , transmission coefficient, usually taken as unity; N , Avogadro's constant; h , Planck constant; $S^\ddagger$, activation entropy; k_B , Boltzmann constant; P , probability factor; d_{AB} , average diameter of the two reacting molecules A and B in cm; m_A and m_B , mass of A and B, respectively; H^0 normal enthalpy; S^0 , normal entropy; $G^\ddagger$ activation free enthalpy; c_A , c_B , and c_{AB} , molar concentrations of A, B and the activated complex, respectively; c_0 , 1 M, taken as reference; v_0 , initial velocity; $V_{\max}$, maximum velocity; E_T , total enzyme concentration; S , substrate concentration; K_s , dissociation constant; K_m , Michaelis constant.

i.e. the pre-exponential factor:

$$A_1^0 = (\kappa RT/Nh) e^{\Delta S^\ddagger/R} = A_1 e^{\Delta S^\ddagger/R} \quad (2)$$

and A_1 is the 'propre-exponential factor'. Both the first order rate constant and A_1 have the dimension of s^{-1} . Consequently, in analysing the temperature dependence of a rate constant graphically either $\log(k/A_1)$ or $\log(k \cdot s)$ should be plotted as a function of $1/T$, since the former are dimensionless quantities. Moreover, in the plot $\log(k \cdot s)$ versus $1/T$ the intercept, A_1^0 , is also the function of T (really of $\log T$) although its temperature dependence is very weak (Benson, 1960). Since this plot is not linear theoretically, the plot $\log[(k/T)(K \cdot s)]$ versus $1/T$ is recommended.

The numerical values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ are correct only if k is given in s^{-1} . Sometimes one finds Arrhenius plots where k is given in min^{-1} or h^{-1} , which may result in erroneous numerical values if it is neglected that A_1 has the dimension s^{-1} . Consequently, in these cases A_1 should be multiplied by 60 or 3600, respectively, to obtain the correct value (cf. Arrhenius, 1889).

Second order reactions. Here the rate constant may be interpreted either in terms of the collision theory (Lewis, 1918) or the absolute rate theory. By using the collision theory one obtains:

$$k = PNd_{AB}^2 [8\pi k_B T (m_A + m_B)/m_A m_B]^{1/2} e^{-E_a/RT} \quad (3)$$

i.e. the pre-exponential factor:

$$A_2^0 = Nd_{AB}^2 [8\pi k_B T (m_A + m_B)/m_A m_B]^{1/2} e^{\Delta S^\ddagger/R} \\ = A_2 e^{\Delta S^\ddagger/R} \quad (4)$$

where A_2 is the 'propre-exponential factor'.

In this case the dimension of A_2 is $\text{cm}^3 \cdot \text{mol}^{-1} \cdot \text{l}^1$. To use dimensionless quantities either $\log(k/10^3 A_2)$ or $\log(k \cdot \text{M} \cdot \text{s})$ should be plotted against $1/T$, since k and $10^3 A_2 = A'_2$ have the same dimensions, i.e. $\text{M}^{-1} \cdot \text{s}^{-1}$.

If $\log k$ (k in $\text{M}^{-1} \cdot \text{s}^{-1}$) versus $1/T$ is plotted, as occurs commonly, the intercept on the ordinate will be $\log A'_2 + \Delta S^\ddagger/2.303R$, which generally is not taken into account (e.g. Thusius *et al.*, 1976; Brandsnes *et al.*, 1982). The error in $\Delta S^\ddagger$ may reach $\pm 100\%$ or even more if $\log A'_2$ is neglected, e.g. for a protein of 150000 Da and a substrate of about 500 Da. Again, if k is given in $\text{M}^{-1} \cdot \text{min}^{-1}$ or $\text{M}^{-1} \cdot \text{h}^{-1}$ or $\text{mm}^{-1} \cdot \text{s}^{-1}$, etc., the values will be in error. If the value of $\Delta S^\ddagger$ is needed, the plot $\log[(k/T^\ddagger)(K^\ddagger \cdot \text{M} \cdot \text{s})]$ versus $1/T$ is recommended, which gives also theoretically a straight line, and d_{AB} , m_A and m_B should be calculated. If the latter values are not known, $\Delta S^\ddagger$ cannot be determined.

If the second order rate constant is interpreted by the absolute rate theory:

$$k = (\kappa RT/Nh) K^* \quad (5)$$

where K^* is the equilibrium constant of the formation of the activated complex in relative concentrations, i.e.:

$$\begin{aligned} K^* &= (c_{AB^*}/c_0)/(c_A/c_0)(c_B/c_0) \\ &= (c_{AB^*}c_0)/(c_Ac_B) \end{aligned} \quad (6)$$

Consequently, in this case substituting the van't Hoff equation for K^* , one obtains:

$$k = (\kappa RT/Nh) e^{\Delta S^\ddagger/R} e^{-\Delta H^\ddagger/RT} \quad (7)$$

i.e. the equation is formally equivalent to that of the first order rate constant (Eyring, 1935) and $\log(k/T)(K \cdot \text{s})$ should be plotted against $1/T$. It is supposed here that only the decomposition of the activated complex limits the rate of the overall reaction. Strictly speaking, if k in $\text{M}^{-1} \cdot \text{s}^{-1}$ is obtained for a bimolecular reaction, the use of the Arrhenius plot tacitly assumes the validity of the collision theory, since A'_2 has this dimension (Laidler, 1963). Otherwise, if k is obtained in s^{-1} , even in the case of a bimolecular reaction the absolute rate theory must be regarded as valid and A_1 should be used as pre-exponential factor in the Arrhenius plot.

If the evaluation of $\Delta S^\ddagger$ is not crucial, the use of the integrated Arrhenius equation (Arrhenius, 1889) is recommended:

$$\log(k_2/k_1) =$$

$$(E_a/2.303R)[(T_2 - T_1)/(T_2 T_1)] \quad (8)$$

where k_2/k_1 is dimensionless, both being rate constants of the same process at two temperatures. It is suggested that k_1 at T_1 should be taken as constant reference values for all k values.

We must draw attention to the fact that very often one finds $\log v_0$ versus $1/T$ presented as an 'Arrhenius plot' (e.g. Coulet *et al.*, 1975; Terra *et al.*, 1978; Lucas, 1978; Bragadin *et al.*, 1979; McMurchie & Raison, 1979; Silviu & McElhaney, 1980; Kwatra & Sourkes, 1981; Rosemeyer *et al.*, 1982). This is a fundamental mistake caused by an inappropriate approximation. Since $v_0 = V_{\max} S/(K_m + S)$, and K_m is a function of the temperature, one cannot use v_0 instead of k , only $V_{\max}/E_T$. It is not by chance that practically in all papers where $\log v_0$ was plotted against $1/T$ a break in the 'Arrhenius plot' was found, which was wrongly interpreted as a change in protein structure since it is due only to the temperature dependence of K_m . One should be aware that by using only $V_{\max}$ instead of $V_{\max}/E_T$ for the Arrhenius plot, the intercept on the ordinate will be affected by the value of $\log E_T$, which may be equal or even higher than $\Delta S^\ddagger$ (Reiner, 1969).

One can use $V_{\max}/E_T$ for the Arrhenius plot if and only if $V_{\max} = k_2 E_T$, i.e. the reaction follows the simple Michaelis-Menten mechanism (Keleti, 1981). If, e.g. a kinetically relevant EP complex is also formed:

and $V_{\max}/E_T$ cannot be used for the Arrhenius plot, since this refers only to simple elementary steps.

van't Hoff plots

(a) In the analysis of the temperature dependence of equilibrium constants, similar misinterpretations are common. If in the reversible reaction no change in mole number occurs

the equilibrium constant is a dimensionless number, i.e. the use of the van't Hoff equation is correct. In the case of first order reactions, as:

$$\begin{aligned} K_{\text{eq}} = k_{-1}/k_{+1} = (A_{-1} e^{-\Delta G^\ddagger_1/RT}) / \\ (A_{+1} e^{-\Delta G^\ddagger_1/RT}) = e^{-\Delta G^0/RT} \end{aligned} \quad (9)$$

no problem arises. Since $A_{+1} = A_{-1}$, $R \ln(A_{-1}/A_{+1}) = \Delta S^0$ (Benson, 1960; Laidler, 1963).

Generally, by plotting $\log K_{\text{eq}}$ versus $1/T$ the slope of the straight line is $-H^0/2.303R$ and the intercept on the ordinate is $\Delta S^0/2.303R$. However, this is true only if the reaction is of first order in both directions.

(b) In the case of second order reactions, if they are to be interpreted by the collision theory (i.e. the k values are in $\text{M}^{-1} \cdot \text{s}^{-1}$), for the elimination of the pre-exponential factors one should assume that $d_{AB}^2 \approx d_{CD}^2$ and $(m_A + m_B)/m_A m_B \approx (m_C + m_D)/m_C m_D$.

In most instances this is probably true. However it is surely not true when, e.g. A is a protein or polysaccharide or polynucleotide and C is a small peptide or mono- or disaccharide or mono- or dinucleotide, etc. In these cases $\Delta G^0 = -RT \ln(A'_{-2}K_{eq}/A'_{+2})$. From $R \ln(A^0_{-2}/A^0_{+2})$ instead of ΔS^0 one obtains $\ln(A'_{-2}/A'_{+2}) + \Delta S^0$, since $\ln(A'_{-2}/A'_{+2})$ differs from zero.

(c) If there is a change in mole number in the reaction at equilibrium

$$K_{eq} = k_2/k_1 = A'_2 e^{-\Delta G^\ddagger_2/RT}/A'_1 e^{-\Delta G^\ddagger_1/RT} \quad (10)$$

since k_1 is a first-order rate constant and k_2 a second-order rate constant.

The dimension of K_{eq} is often (but wrongly, since it should be dimensionless; cf. Price & Dwek, 1979) presented as M^{-1} (e.g. Schweitz *et al.*, 1973; Vincent & Lazdunski, 1973; Luisi *et al.*, 1975) or M (e.g. Burton, 1974; Hochachka, 1974; Nowak & Lee, 1977; Kozlov *et al.*, 1977; Schultz *et al.*, 1977; Hofmann *et al.*, 1979; Takahashi *et al.*, 1981). The right equation to be used is:

$$\Delta G^0 = -RT \ln(A_1 K_{eq}/A'_2) \quad (11)$$

since $A_1 K_{eq}/A'_2$ is a dimensionless quantity and surely $A_1 \neq A'_2$. If one analyses the temperature dependence of the equilibrium constant of a reversible enzyme-substrate or enzyme-inhibitor complex formation, d^2_{ES} or d^2_{EI} may be assumed to equal d^2_E . With a protein of about 150 000 Da ($d \approx 7$ nm) and a substrate or inhibitor of about 500 Da, A'_2 at 300 K is several orders of magnitude higher than A_1 , the latter being about $6 \times 10^{12} \text{ s}^{-1}$. The same reasoning is valid for receptor-ligand reactions.

By plotting $-\log K_{eq}$ versus $1/T$ the intercept on the ordinate will be $\Delta S^0/2.303R + \log(A_1/A'_2)$. One should be aware that also $\log(A_1/A'_2)$ is a function of T [really of $\log(1/T^\ddagger)$] (Lewis, 1918), even if its temperature-dependence is very weak. Therefore it is better to plot $\log[(K_{eq}T^\ddagger)(M \cdot K^{-\ddagger})]$ versus $1/T$, which also gives a straight line theoretically. Again the concentrations of the compounds in the expression of K_{eq} should be expressed in M and not in mM , μM , etc., if the second order rate constant is interpreted by the collision theory.

Since in such cases ΔS^0 generally cannot be evaluated with the desired accuracy, the use of the integrated van't Hoff equation (van't Hoff, 1884; Arrhenius, 1889) is recommended:

$$\log(K_{eq,2}/K_{eq,1}) = (\Delta H^0/2.303R)(1/T_2 - 1/T_1) \quad (12)$$

$K_{eq,1}$ at T_1 being taken as reference for all K_{eq} values.

(d) If one can interpret also the second-order reaction in the reversible one by the absolute rate

theory, i.e. k values are in s^{-1} in both directions, the equilibrium constant of a reaction in which a change in mole number occurs will be:

$$K_{eq} = k_2/k_1 = e^{-\Delta G^0/RT}$$

since

$$K_{eq} = (c_A/c_0)/(c_C/c_0)(c_D/c_0) = (c_A c_D)/(c_C c_D) \quad (13)$$

is a dimensionless number, as it should be (Price & Dwek, 1979), and the pre-exponential factors in s^{-1} are equal.

(e) Owing to the arguments presented above, the determination of ΔG^0 of enzyme-substrate complex formation using a plot of $\log K_m$ versus $1/T$ has no meaning. Either $K_m = K_s$ and in this case point (c) above is valid, or $K_m = (k_{-1} + k_{+2})/k_1 = K_s + k_{+2}/k_{+1}$, which is not interpretable according to the van't Hoff equation. If the mechanism of the reaction is more complex than the simple Michaelis-Menten mechanism, similar plots are even more meaningless (e.g. Tro & Keleti, 1974).

General conclusions

The above statements are independent of the phenomenological interpretation of the pre-exponential factors; the latter were involved only to help elucidate the general misinterpretations.

The use of $k \cdot s$ or $(k/T)(K \cdot s)$, etc. seems to be simply a formal elimination of the logarithmization of dimensioned quantities. However, it is the only way to ensure that dimensional inequalities do not lead to erroneous numerical values for the thermodynamic parameters.

We suggest to re-evaluate the $\Delta S^\ddagger$, $\Delta H^\ddagger$, $\Delta G^\ddagger$, ΔH^0 , ΔS^0 and ΔG^0 values calculated without taking into account the problems outlined above. However, it should be considered that in second-order reactions the numerical values of $\Delta S^\ddagger$ and ΔS^0 , and consequently those of $\Delta G^\ddagger$ and ΔG^0 , are questionable, due to the difficulties of evaluating d_{AB} , m_A , m_B and c_{AB^*} .

The ΔG^0 values generally cannot be used to compare the efficiencies of metabolic processes, since these often involve change in mole number and therefore relative concentrations for the van't Hoff plot should be applied (Cornish-Bowden, 1981).

It should be noted that there are a great number of papers which contain similar errors. I apologize for referring to the authors mentioned, chosen by chance, instead of several dozen (or hundred) others which could be selected equally.

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